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Crystallization and preliminary crystallographic studies of FMN-binding protein from *Desulfovibrio vulgaris* Miyazaki F

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The flavin mononucleotide binding protein from *Desulfovibrio vulgaris* (Miyazaki F) was crystallized using the vapour-diffusion method. The crystal belongs to the monoclinic space group $P2_1$ with unit-cell parameters a = 37.2, b = 84.6, c = 41.1 Å, β = 94.1°, contains two molecules per asymmetric unit and diffracts beyond 1.2 Å resolution with a synchrotron radiation X-ray source.

1. Introduction

Sulfate-reducing bacteria possess many redox proteins including some flavoproteins. A gene encoding a novel flavin mononucleotide binding protein (FMN-bp) from *Desulfovibrio vulgaris* (Miyazaki F) was cloned and its expression system was constructed in *Escherichia coli* (Kitamura *et al.*, 1994). Immunoblot analysis of the whole proteins from *D. vulgaris* (Miyazaki F) indicated that this protein also exists in the bacterium. FMN-binding protein is composed of 122 amino acids, which is the lowest number of residues in known FMN-binding proteins. The function of FMN-bp *in vivo* is at present unclear, although it might take part in the electron-transfer pathway. Flavodoxins are a group of proteins with a tightly bound FMN which mediate electron transfer at low redox potential. Flavodoxins are well known to exist in three oxidation states, namely the oxidized form, the semiquinone form and the hydroquinone form. The crystal structures of flavodoxin in these oxidation states have been reported (Watt *et al.*, 1991). The FMN-bp from *D. vulgaris* (Miyazaki F) does not have any homology with other proteins which bind FMN, including flavodoxin. In addition, the semiquinone state of FMN-bp is unstable, whereas that of flavodoxin is stable. FMN-bp has a similar molecular weight to that of flavodoxin. To elucidate the reasons for the differences in the oxidation state stabilities of FMN-bp and flavodoxin, the structures of FMN-bp in two states at high resolution are required.

The structural study of FMN-bp using nuclear magnetic resonance spectroscopy has previously been reported and FMN-bp is suggested to have a structural relationship to a precursor of chymotrypsin (Liepinsh *et al.*, 1997). According to the structure analyzed by NMR and considering FMN-bp to be a monomer, the FMN-bp molecule has an exposed hydrophobic surface area. In addition, the molecular weight of FMN-bp was estimated to be 17500 Da using the gel-filtration method (Kitamura *et al.*, 1994), which is higher than the calculated value of 13700 Da. These observations may imply that FMN-bp exists in a dimeric form, at least partly, and the dimeric structure may provide new information towards the understanding of the protein function. From this point of view, the structure determination of FMN-bp in the crystalline state is considered to be necessary in addition to the solution structure previously determined by NMR spectroscopy.

2. Experimental and results

FMN-bp was purified as described previously (Kitamura *et al.*, 1994). The protein solution contained 15 mg ml^{-1} FMN-bp, 0.1 *M* Tris–HCl (pH 8.0) and 200 m*M* sodium chloride, in which FMN-bp is in the oxidized form.

We first attempted to crystallize FMN-bp using ammonium sulfate as a precipitant, and crystals were obtained. Though they did not grow to a size suitable for X-ray study, they were used as seeds in subsequent experiments. Single crystals were grown using polyethylene glycol using the vapour-diffusion method with

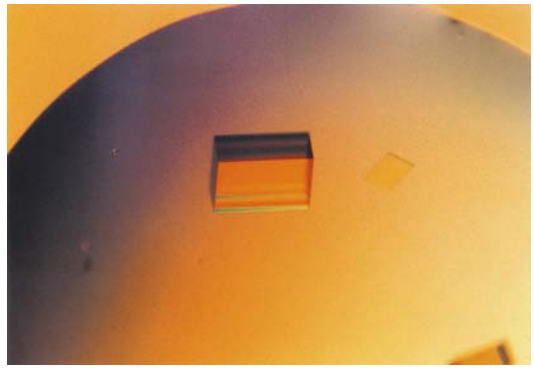

Figure 1
Crystals of FMN-bp from *D. vulgaris* (Miyazaki F). The largest crystal is approximately 0.7 × 0.5 × 0.2 mm.

Table 1
Summary of data collections.

The crystal of native 1 was produced at pH 6.5, while the native 2 crystals were produced at pH 7.5.

	Native 1	Native 2
X-ray source	PF (BL-6A)	PF (BL-18B)
Wavelength (Å)	1.00	1.00
Number of crystals	1	2
Number of imaging plates	20	31
Total number of reflections	112414	337547
Number of unique reflections	22986	68514
Space group	$P2_1$	$P2_1$
a (Å)	37.4	37.2
b (Å)	84.4	84.6
c (Å)	41.1	41.1
β (°)	94.2	94.1
Resolution (Å)	30.0–1.60	30.0–1.20
Completeness (%)	68.7	86.6
$R_{\rm merge}$†	4.8	2.6

† $R_{\rm merge} = \sum_{hkl,i} |I_{hkl,i} - \langle I_{hkl}\rangle| / \sum_{hkl,i} I_{hkl,i}$.

a microseeding technique. The reservoir contained 0.1 *M* buffer solution (MES pH 6.5 or Tris–HCl pH 7.5), 20%(*w*/*v*) polyethylene glycol 6000 or 8000 and 0.2 *M* sodium acetate. The protein drops were prepared by mixing 7 µl protein solution with 7 µl reservoir solution before adding the microseeds. Single yellow crystals appeared as thick plates or parallelepipeds within a week, but they were too weak to be put into capillaries and only diffracted to 4 Å. The quality of the crystals was dramatically improved (Fig. 1) by the addition of 20%(*v*/*v*) glycerol to the reservoir solution (Sousa, 1995). The crystals grew to dimensions 0.7 × 0.5 × 0.3 mm in two weeks.

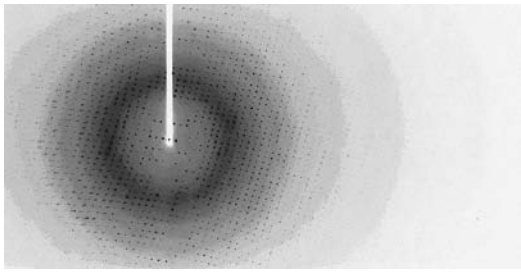

Figure 2
The diffraction image collected at the BL41XU beamline, SPring-8, Japan.

The diffraction intensity data were collected using synchrotron radiation at the BL-6A and BL-18B beamlines, Photon Factory, KEK, Japan (Table 1). Weissenberg cameras for macromolecular crystallography (Sakabe, 1983) were used for native 1 and 2 data sets. Small imaging plates (20 × 40 cm) were used for the native 1 data set and large imaging plates (40 × 80 cm) (Sakabe *et al.*, 1997) were used for the native 2 data set. The X-ray wavelength used was 1.0 Å. All diffraction experiments were carried out at room temperature. The data set of native 1 was collected over 90° with rotation along the a^* axis, while native 2 was collected over 90° with rotation along the a^* and c^* axes.

The programs *DENZO* and *SCALEPACK* (Otwinowski, 1993) were used for data processing. For the individual data sets, the overall $R_{\rm merge}$ was 2.6–4.8% and $R_{\rm merge}$ for the highest shell was less than 20% with $I \geq 1\sigma(I)$. All crystals belong to the monoclinic space group $P2_1$. Assuming that two molecules are present in the asymmetric unit, the V_m values were within the range 2.35–2.36 Å^3 Da^{-1}, which is reasonable for protein crystals (Matthews, 1968).

Because the crystals of FMN-bp diffract to high resolution, their resolution limits tend to depend on the camera parameters such as camera distance, size of imaging plates *etc.* For example, they diffract beyond 1.0 Å using X-rays of wavelength 0.708 Å at SPring-8, as shown in Fig. 2. The native 2 data set could be merged with a very low $R_{\rm merge}$ value despite using two crystals which were prepared from the same purified sample but were not from the same batch.

A search for heavy-atom derivatives is currently under way to enable structure determination by the isomorphous replacement method.

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